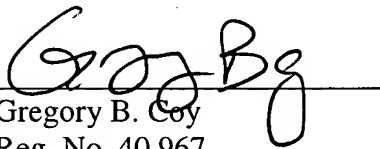


REMARKS

In accordance with the suggestion of Examiner Collins during a telephone conference with the undersigned on April 29, 2004, the purpose of this Supplemental Response is to transmit a signed Declaration Under 37 C.F.R. §1.132, including an attached curriculum vita of Dr. Frank J. Turano.

On April 20, 2004, an Amendment After First Office Action was submitted to the PTO in this case, along with an unsigned Declaration Under 37 C.F.R. §1.132, which had been prepared for signature by Frank J. Turano, Ph.D. Dr. Turano participated in the preparation of the Declaration and had approved the text of the Declaration; however, due to unforeseen circumstances, Dr. Turano was not able to sign the Declaration prior to its submission to the PTO. The Declaration was therefore submitted to the PTO unsigned. In addition, the unsigned Declaration referenced Dr. Turano's curriculum vita, which was not submitted with the unsigned Declaration on April 20, 2004. The Declaration has now been signed by Dr. Turano and the signed Declaration is submitted herewith along with Dr. Turano's curriculum vita. Please consider these documents along with the Amendment after First Office Action submitted on April 20, 2004.

Respectfully submitted:

By: 
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SUPPLEMENTAL RESPONSE TO FIRST ACTION

Serial No. 10/006,852

Attorney Docket No. 7224-65

Page 2 of 2

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re patent application of:

Alan M. Kinnersley et al.

Serial No. 10/006,852

Filed November 7, 2001

METHODS FOR REGULATING PLANT
GABA PRODUCTION

)
) Before the Examiner
) Cynthia Collins
)
)
) Group Art Unit 1638
)
)
)
)

DECLARATION UNDER 37 C.F.R. §1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Frank J. Turano, hereby declare that:

1. I am a named inventor on the above-captioned patent application and I am familiar with its content.

2. My degrees include a M.S. degree in Environmental Sciences and a Ph.D. in Botany from Miami University of Ohio. I have significant experience in plant research and I currently hold the position of Associate Professor. I have memberships in The American Society of Plant Biologists, International Society for Plant Molecular Biology, Mid-Atlantic Plant Molecular Biology Society, and Mid-Atlantic Section of the American Society of Plant Biologists. I have authored or co-authored twenty-three publications, book chapters, or review articles; have made forty seminar presentations; and have been awarded seven (five as Principal Investigator and two as Co-Principal Investigator) grants, all in the field of the present invention. I have served as a reviewer or referee for five international and national journals. I have been a reviewer for national and international grant committees. Further information relating to my education and experience in this field is provided in my *curriculum vita*, a copy of which is attached hereto.

DECLARATION UNDER 37 C.F.R. §1.132

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3. I have reviewed the Office Action dated November 20, 2003 in the above-captioned patent application. In the outstanding Office Action, claims 1-15, 19-20, 22-23, 26-28 and 31-40 are rejected under 35 U.S.C. §112, first paragraph, as failing to comply with the written description requirement. The Examiner states in the Action that, "The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention." The Examiner's statements in the Action in support of this position suggest that she believes each of the pending claims either lacks sufficient structural definition or lacks sufficient functional definition. In reply, based upon my training and experience, and for the reasons discussed below, it is my opinion that a person of ordinary skill in the art would interpret the claimed subject matter as being structurally and functionally defined with a significant degree of particularity.

4. GAD enzymes were well known in the literature at the time the present patent application was filed. The very statement of the name "GAD" at that time conveyed a significant amount of information to a person of ordinary skill in the art regarding structural and functional characteristics of the GAD enzyme. The Examiner includes in the outstanding Action a summary of several structural characteristics of functional GAD enzymes described in one of my prior publications. An excerpt from page 5 of the outstanding Action, which refers to my publication, is set forth below:

For example, Turano et al. teach that GAD peptides are divided into 3 distinct regions: (1) a small amino terminal variable region of unknown functional significance, (2) a largely conserved middle region encoding GAD enzymatic [sic] activity, and (3) a small carboxy terminal variable region encoding a calmodulin binding domain. Turano et al. also teach that the Arabidopsis GAD1 and GAD2 sequences comprise a Ser-X-X-Lys amino acid motif common among PLP-requiring enzymes, and that the Ser-X-X-Lys motif is conserved in both identity and position as compared to GAD enzymes of petunia, tomato, and the *gadA* and *gadB* genes of *E. coli*. (Citation omitted).

At the time the present application was filed, persons of ordinary skill in the art knew the structural characteristics discussed in the above excerpt. A person of ordinary skill in the art, upon considering the language of the pending claims, each of which recites a "GAD enzyme" would at that time have immediately understood the structural features that are known to be

present in functional GAD enzymes. The claims therefore recite sufficient structural definition. A person of ordinary skill in the art would immediately recognize that the claims properly describe the invention in terms of structure and function, and that Applicants, at the time the application was filed, were in possession of the claimed invention.

5. The specification describes a representative number of examples of GAD enzymes encompassed by the claims, i.e., examples that are representative of the full scope of the claims. In this regard, it is stated in the Action that, “the specification describes only nine specific sequences obtained from four different species of dicotyledonous plants (Arabidopsis, tobacco, petunia and tomato) that are said to correspond to GAD enzymes.” (emphasis added) It is apparent, however, upon proper consideration of the breadth of the claimed subject matter, that the description of nine (9) specific sequences far exceeds a “representative number of species” and is much more than a minimum number necessary to support the claimed genus. It is also stated in the outstanding Action that, “the specification does not describe the extent to which, if any, these [nine] sequences are structurally and functionally related to one another.” In reply, I would again note that a person of ordinary skill in the art would readily understand the structural features of a GAD enzyme, would readily identify the sequences set forth in the Sequence Listing as GAD enzymes, and would be readily able to determine the level of structural similarity between the sequences in the Sequence Listing, particularly in view of the guidance provided in the specification regarding determining sequence identity.

6. Set forth below is information showing the structural relatedness of the nine sequences in terms of percent sequence identity. The identified sequences were compared to one another using the MacVector program and the default parameters set forth at page 23 of the present specification.

Amino acid sequence identities

SEQ 2: Arabidopsis thaliana GAD1	-and-	SEQ 4: Arabidopsis thaliana GAD2	= 80.9%
SEQ2: Arabidopsis thaliana GAD1	-and-	SEQ 6: Arabidopsis thaliana GAD3	= 78.7%
SEQ2: Arabidopsis thaliana GAD1	-and-	SEQ 8: Arabidopsis thaliana GAD4	= 81.7%
SEQ2: Arabidopsis thaliana GAD1	-and-	SEQ 10: Arabidopsis thaliana GAD5	= 74.5%

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SEQ2: Arabidopsis thaliana GAD1	-and-	SEQ 12: Tobacco NtGAD1	= 85.3%
SEQ2: Arabidopsis thaliana GAD1	-and-	SEQ 14: Tobacco NtGAD2	= 84.3%
SEQ2: Arabidopsis thaliana GAD1	-and-	SEQ 16: Petunia GAD	= 85.0%
SEQ2: Arabidopsis thaliana GAD1	-and-	SEQ 18: Tomato GAD	= 76.1%
<hr/>			
SEQ4: Arabidopsis thaliana GAD2	-and-	SEQ 6: Arabidopsis thaliana GAD3	= 77.2%
SEQ4: Arabidopsis thaliana GAD2	-and-	SEQ 8: Arabidopsis thaliana GAD4	= 80.0%
SEQ4: Arabidopsis thaliana GAD2	-and-	SEQ 10: Arabidopsis thaliana GAD5	= 74.1%
SEQ4: Arabidopsis thaliana GAD2	-and-	SEQ 12: Tobacco NtGAD1	= 81.0%
SEQ4: Arabidopsis thaliana GAD2	-and-	SEQ 14: Tobacco NtGAD2	= 80.0%
SEQ4: Arabidopsis thaliana GAD2	-and-	SEQ 16: Petunia GAD	= 82.0%
SEQ4: Arabidopsis thaliana GAD2	-and-	SEQ 18: Tomato GAD	= 75.1%
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SEQ6: Arabidopsis thaliana GAD3	-and-	SEQ 8: Arabidopsis thaliana GAD4	= 91.2%
SEQ6: Arabidopsis thaliana GAD3	-and-	SEQ 10: Arabidopsis thaliana GAD5	= 73.8%
SEQ6: Arabidopsis thaliana GAD3	-and-	SEQ 12: Tobacco NtGAD1	= 82.6%
SEQ6: Arabidopsis thaliana GAD3	-and-	SEQ 14: Tobacco NtGAD2	= 81.2%
SEQ6: Arabidopsis thaliana GAD3	-and-	SEQ 16: Petunia GAD	= 82.2%
SEQ6: Arabidopsis thaliana GAD3	-and-	SEQ 18: Tomato GAD	= 72.8%
<hr/>			
SEQ 8: Arabidopsis thaliana GAD4	-and-	SEQ 10: Arabidopsis thaliana GAD5	= 76.7%
SEQ 8: Arabidopsis thaliana GAD4	-and-	SEQ 12: Tobacco NtGAD1	= 85.9%
SEQ 8: Arabidopsis thaliana GAD4	-and-	SEQ 14: Tobacco NtGAD2	= 84.3%
SEQ 8: Arabidopsis thaliana GAD4	-and-	SEQ 16: Petunia GAD	= 85.4%
SEQ 8: Arabidopsis thaliana GAD4	-and-	SEQ 18: Tomato GAD	= 74.5%

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SEQ 10: Arabidopsis thaliana GAD5	-and-	SEQ 12: Tobacco NtGAD1	= 76.8%
SEQ 10: Arabidopsis thaliana GAD5	-and-	SEQ 14: Tobacco NtGAD2	= 75.6%
SEQ 10: Arabidopsis thaliana GAD5	-and-	SEQ 16: Petunia GAD	= 77.6 %
SEQ 10: Arabidopsis thaliana GAD5	-and-	SEQ 18: Tomato GAD	= 75.1 %

The structural and functional relatedness of the sequences would have been apparent to a person of ordinary skill in the art upon consideration of the specification at the time the present application was filed.

7. It is also stated in the Action that, “the specification does not describe the structural characteristics of a modified GAD enzyme that does not include a functional autoinhibitory calmodulin-binding domain, even though the prior art indicates that specific but variable structural characteristics as [sic] associated with the calmodulin-binding domain of plant GAD enzymes.” Based upon my training and experience in this field, I believe that a modified GAD enzyme without a functional autoinhibitory calmodulin-binding domain is described in the specification in a manner that would be understood by a person of ordinary skill in the art. Calmodulin-binding domains of plant GAD enzymes are located in the carboxyterminal region of GAD, and the calmodulin-binding domain varies between different GAD sequences; however, this does not negate the fact that a person of ordinary skill in the art at the time the application was filed would have understood and recognized a calmodulin-binding domain as such. Indeed, given the high degree of conservation in the enzymatic domain of GAD, a person of ordinary skill in the art would have readily recognized the enzymatic portion of a GAD sequence, and concluded that non-conserved residues at the carboxy-terminal end can be excised if it is desired to express a GAD without the calmodulin-binding domain. Thus, based upon the present specification and information available in the art at the time the application was filed, a person of ordinary skill in the art would have known how to make and use the various aspects of the invention relating to GAD enzymes lacking a calmodulin-binding domain.

8. I have also reviewed and considered the rejection in the outstanding Office Action of claims 1-15, 19-20, 22-23, 26-28 and 31-40 under 35 U.S.C. §112, first paragraph,

based upon an assertion that the specification, while being enabling for multiple aspects of the invention as they relate to the GAD2 polynucleotide and GAD2 enzyme of SEQ ID NOS:3 and 4, does not reasonably provide enablement for various aspects of the invention as they relate to other GAD enzymes, such as the elected plant GAD enzyme of SEQ ID NO:2. The Examiner states in the Action that, "The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims."

9. GAD enzymes featuring the sequences set forth in the Sequence Listing have been shown in the literature to be functional to catalyze the conversion of glutamate to GABA. In this regard, several of the plant GADS set forth in the application have been successfully expressed as functional recombinant proteins in diverse species such as *E. coli*, insect cell lines and plants (Baum et al. *J. Biol. Chem.*, 15 Sept. 1993, Vol. 268, No. 26, pages 19610-19617; Baum et al., *EMBO J.*, 17 June 1996, Vol. 15, No. 12, pages 2988-2996; Snedden et al., *J. Biol. Chem.*, 23 Feb. 1996, Vol. 271, No. 8, pages 4148-4153; Turano and Fang, *Plant Physiol.*, Aug. 1998, Vol. 117, No. 4, pages 1411-1421; Mclean et al., *Molecular breeding: new strategies in plant improvement*. May 2003, Vol. 11, No. 4, pages 277-285 2003; MacGregor et al. 2003 *J Chem Ecol.* 2003 Sep;29(9):2177-21782)). Sequences having these degrees of primary sequence similarity have been shown to have the desired functional activity, i.e., catalyzing the GAD reaction, which is the conversion of glutamate to GABA.

10. The following is stated in the outstanding Office Action:

[T]he specification only discloses the phenotypes of plants transformed with polynucleotides encoding one type of GAD enzyme, the *Arabidopsis* GAD2 (nonelected SEQ ID NO:4) enzyme... The specification does not disclose the phenotypes of plants transformed with polynucleotides encoding other GAD enzymes, such as the *Arabidopsis* GAD1 (elected SEQ ID NO:2) enzyme, or GAD enzymes ... having at least about 60% identity to [SEQ ID NO:2] or by sequences that hybridize to SEQ ID NO:1 under moderately stringent conditions.

It is further stated in the action that the absence of this information defeats the enablement of the claims because, "the effect on transgenic plants of expressing a GAD enzyme, with or without a calmodulin-binding domain, at different levels or at different times or in different

locations or under different conditions is unpredictable.” (Office Action, page 10). The Action then states the following two reasons that the effect is unpredictable:

- (1) “The effect is unpredictable because different levels of GAD and its product GABA have different effects on plants.” (citations omitted) (Office Action, page 10); and
- (2) “The effect on transgenic plants of expressing a GAD enzyme is also unpredictable because the level of GAD expression and GAD activity would be affected by multiple variables which include but are not limited to whether the GAD enzyme retained its calmodulin binding domain, the type of promoter and terminator used in the expression vector, the plant species transformed by the expression vector, the type of tissue in which GAD is expressed, the stability of the mRNA transcribed from the GAD coding sequence, the translation efficiency of the mRNA, GAD stability, the availability of glutamate substrate and other substances, such as calcium and calmodulin and PLP, that would affect GAD activity.” (citations omitted) (Office Action, page 11).

In view of the above, the Examiner concludes that the full scope of the claimed invention is not enabled because:

- (1) “[T]he specification does not provide sufficient guidance for one skilled in the art to determine, without undue experimentation, which combinations of GAD enzymes and non-constitutive promoters would result in a level of GAD expression and/or activity that would produce a specific desired phenotypic effect in plants transformed therewith.” (Office Action, page 11); and
- (2) “[T]he specification does not provide sufficient guidance for one skilled in the art to determine, without undue experimentation, how to express a GAD1 enzyme in a manner that would produce specific phenotypic effects comparable to those produced in plants transformed with constructs comprising GAD2 sequences.” (Office Action, page 11).

11. The undersigned does not dispute that the field of the present invention can be characterized as unpredictable; however, based upon my training and experience in this field, I believe that the level of unpredictability associated with the present invention is no greater than that typical of performing any plant genetic transformation, and that the claimed invention does not require undue experimentation. It is important to understand that biological systems by their nature include variability, and those skilled in the art understand that genetic transformation events produce variable results. In other words, due to the well-documented

phenomena of epigenetic genetics (Qin et al. 2003 *Plant Mol Biol.* 2003 May;52(1):217-231; Meyer, 2000 *Plant Mol Biol.* 2000 Jun;43(2-3):221-234; Meyer 2003 *Semin Cell Dev Biol.* 2003 Feb;14(1):51-52; Flavell, 1998 *Novartis Found Symp.* 1998; 214: 144-154); and position effects (van Leeuwen et al. 2001.*Plant Mol Biol.* 2001 Nov;47(4):543-54; Matzke AJ, Matzke MA. *Curr Opin Plant Biol.* 1998 Apr;1(2): pages 142-148) when making transgenic plant lines, a plant transformation process is inherently variable. Notwithstanding this variability, a person of ordinary skill in the art, upon reading the present specification, would understand that excellent results of the invention can be achieved by over-expressing GAD in a plant in a manner whereby increased levels of GABA are produced, but whereby GABA is not overproduced at a level whereby the plant is stunted or sterile or otherwise has undesirable morphological characteristics. In other words, the present specification clearly teaches to a person of ordinary skill in the art that transformation of a plant in accordance with the invention to achieve controlled enhancement of GABA production, i.e., GABA production at a higher level than a wild-type plant, but not so great as to produce stunting, sterility and the like, provides desired characteristics in the plant or plant tissues. When this information is considered together with the well-known identity and functionality of GAD enzymes, and the showing in the specification that a plant transformed with a GAD construct indeed had the desired characteristics, a person of ordinary skill in the art would readily appreciate that the present specification enables a skilled artisan to transform plants with functional GAD enzymes. A person of ordinary skill in the art would further appreciate that, due to the inherent variability associated with plant transformation events, it is appropriate to make multiple transformed plants (preferably 25-50 or more) with a given construct, and then to select one or more plants that over-expresses the functional GAD enzyme at a desired level, i.e., plants that do not over-express to a level that causes stunting or sterility or the like. While the specification includes data relating to plants transformed with one GAD construct, a person of ordinary skill in the art would reasonably expect to be enabled to practice the full scope of the claimed invention, including use of constructs including other functional GAD enzymes, in view of the descriptions included in the specification.

12. I have reviewed and considered the rejection in the outstanding Office Action of claims 8, 12, 28, 31, 32, 34, 35, 38 and 40 under 35 U.S.C. §112, second paragraph, as being

indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. With reference to claims 12, 35 and 40, the Examiner states that these claims are indefinite in the recitation of “moderately stringent conditions.” In support of this rejection, the Examiner states that, “It is unclear what type of hybridization conditions would be ‘moderately’ stringent. Neither the claims nor the specification indicate specific hybridization conditions that are ‘moderately’ stringent. Furthermore, those skilled in the art would interpret ‘moderately’ differently.”

13. The specification at pages 22-23 includes the following statements, which define “moderately stringent conditions” in a manner that would be readily understood by a person of ordinary skill in the art:

In another embodiment, the polynucleotide has a sequence that encodes a functional plant GAD enzyme, and has a sequence sufficiently similar to the coding region of a reference polynucleotide that it will hybridize therewith under moderately stringent conditions. This method of determining similarity is well known in the art to which the invention pertains. Briefly, moderately stringent conditions are defined in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd ed. Vol. 1, pp. 101-104, Cold Spring Harbor Laboratory Press (1989) as including the use of a prewashing solution of 5X SSC (a sodium chloride/sodium citrate solution), 0.5% sodium dodecyl sulfate (SDS), 1.0 mM ethylene diaminetetraacetic acid (EDTA) (pH 8.0) and hybridization and washing conditions of 55°C, 5x SSC.

In view of this description in the specification, a person of ordinary skill in the art would understand the meaning of “moderately stringent conditions” to be clear and definite, and would readily be able to determine if a given sequence satisfies this requirement.

14. I have reviewed and considered the assertions in the outstanding Office Action that claims 31-40 are rejected under 35 U.S.C. §102(b) as being anticipated by Baum et al. (EMBO J., 17 June 1996, Vol. 15, No. 12, pages 2988-2996). Claim 31 has now been amended and this claim, as amended, recites subject matter that is clearly novel over Baum et al. The only transformed plant described by Baum et al. that expressed a GAD enzyme not including a functional autoinhibitory calmodulin-binding domain (i.e., a “de-regulated” GAD) exhibited significant loss of growth characteristics, yield, reproductive function or other morphological or agronomic characteristic compared to a non-transformed plant. (See Baum et al. page 2989, column 1, first full paragraph). Morphological features of this type are

expressly excluded by Applicants' claim 31, which recites methods involving transformed plants that over-produce GABA at a level whereby these undesirable phenotypes do not result. The present invention involves the recognition that, although excessive overproduction of GABA in a plant causes stunting and other undesirable agronomic and/or morphological characteristics, non-excessive overproduction of GABA in a plant results in beneficial characteristics, such as, for example, enhanced stress resistance or other desirable morphological and/or agronomic characteristics. (See specification, page 13, lines 16-21). A desirable level of overproduction of GABA is achieved in the method of claim 31, as amended, by transforming a plant that constitutively expresses a de-regulated GAD enzyme, and then selecting a plant that does not exhibit stunting, sterility and/or other undesirable characteristics that are indicative of excessive overproduction of GABA. This method is not anticipated by any method described in the cited Baum et al. reference.

15. The undersigned has read, and understands, the assertions in the outstanding Office Action that claims 1-15, 19-20, 22-23 and 26-27 are rejected under 35 U.S.C. §103(a) as being unpatentable over Baum et al. (EMBO J., 17 June 1996, Vol. 15, No. 12, pages 2988-2996) in view of McKenzie et al. (Plant Physiology, March 1998, Vol. 116, No. 3, pages 969-977). The undersigned also understands that the Examiner can only make this assertion if he or she can establish that a person of ordinary skill in the art would find a teaching, suggestion or motivation in the cited references or elsewhere in the prior art to combine the references in the manner suggested in the outstanding Action. Based upon my training and experience in this field, it is my opinion that there is no identification in the outstanding Office Action of any motivation in the references, or in the literature as a whole, to combine the references in the manner suggested in the Action.

16. In the outstanding Action, the Examiner notes that Baum et al. describe tobacco plants transformed with a vector comprising a constitutive CaMV 35S promoter operably linked to a polynucleotide that encodes a wild type petunia plant GAD enzyme, and tobacco plants transformed with a vector comprising a constitutive CaMV 35S promoter operably linked to a polynucleotide that encodes a mutant petunia plant GAD enzyme that lacks a calmodulin-binding domain. The Examiner also relies upon McKenzie et al. as follows:

McKenzie et al. teach the use of a copper controllable root specific promoter to selectively increase the production of the growth affecting compound cytokinin in response to a signal in plants transformed with a DNA construct comprising a non-constitutive promoter operably linked to a polynucleotide encoding isopentenyl transferase. The plants taught by McKenzie et al. exhibited phenotypic effects associated with cytokinin (loss of apical dominance and delayed leaf senescence), but they did not exhibit the morphological abnormalities exhibited by plants transformed with DNA constructs comprising a constitutive promoter operably linked to a polynucleotide encoding isopentenyl transferase. (Citations omitted).

In support of this rejection, the Examiner states that:

[I]t would have been *prima facie* obvious to one skilled in the art at the time the invention was made to express in a transgenic plant a functional plant GAD enzyme as taught by Baum et al. using non-constitutive promoter [sic] such as the copper controllable root specific promoter taught by McKenzie et al., for the purpose of controlling the phenotypic effect associated with the growth affecting properties of GAD enzyme activity by controlling the time and/or location of GAD enzyme expression, without any surprising or unexplained results. (Office Action, Page 19).

17. None of the statement in the outstanding Office Action identifies any motivation in the cited references or any other prior art to combine the references as suggested in the Action. There is no direct suggestion in either reference to combine their teachings, and there is also no indirect suggestion that could be considered to be a motivation to combine the teachings of the references. Indeed, rather than providing any motivation to combine the teachings of the references as suggested by the Examiner, the Baum et al. reference would actually discourage a person of ordinary skill in the art from performing work that would lead to the present invention. The results of the Baum et al. work would be summarized by a person of ordinary skill in the art as follows:

- (1) plants transformed with a normal GAD (with calmodulin-binding domain) under the control of a constitutive promoter do not have increased GABA levels *in vivo* (see Baum et al., page 2993, column 2, first partial paragraph), and have morphology indistinguishable from that of wild-type plants; and
- (2) plants transformed with a mutant GAD lacking a calmodulin-binding domain under the control of a constitutive promoter exhibit above-normal GABA levels *in vivo* (see Baum et al., page 2993, column 2, first

partial paragraph), and are stunted, sterile, and featured other undesirable morphologic characteristics.

18. The message from this reference to a person of ordinary skill in the art at the time the present application was filed is that elevation of the GABA level in a plant by overexpression of de-regulated GAD is undesirable, and that transformation of a plant with a de-regulated GAD is therefore undesirable. The reference also suggests that transformation of a plant with a normal GAD (i.e., a GAD including a functional calmodulin-binding domain) has no effect on the GABA levels in the plant or the morphology of the plant, and therefore provides no benefit to the plant. Thus, a person of ordinary skill in the art would find no motivation in Baum et al. to transform a plant with a normal GAD or a de-regulated GAD under the control of a non-constitutive promoter, because he or she would expect the resulting plant to be either unchanged morphologically (if including the calmodulin-binding domain) or alternatively to respond to a signal by producing harmful, and perhaps lethal levels of GABA in the tissues expressing the de-regulated GAD (if not including a functional calmodulin-binding domain).

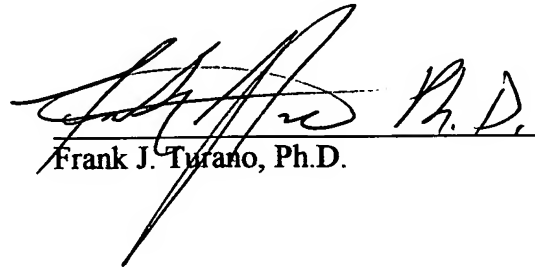
19. In view of the above, and based upon my training and experience in the relevant field, I believe that there is no suggestion in the references of record that there would be any desirable result from overexpressing GAD or overproducing GABA in plant tissues. Without such a suggestion or motivation, there is no suggestion or motivation to combine the two references cited in the outstanding Action.

20. I further declare that all statements made herein are of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date

4/30/04

Frank J. Turano, Ph.D.

 Ph.D.

DECLARATION UNDER 37 C.F.R. §1.132

Serial No. 10/006,852

Attorney Docket No. 7224-65

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Experience

- 1999 - Present Associate Professor, Department of Biological Sciences and Genetics, The George Washington University, Washington, DC
- 1990 - 1999 Plant Molecular Biologist, (Principal Investigator) USDA, Climate Stress Laboratory, Beltsville, MD
- 1986 - 1990 Research Geneticist, (post-doctoral fellow) USDA, Plant Molecular Biology Laboratory, Beltsville, MD
- 1981 - 1986 Dissertation Fellow and Teaching Assistant, Miami University, Department of Botany, Oxford, OH

Other Positions and Experience

- 2003 - Present Director, Howard Hughes Undergraduate Research Program, Department of Biological Sciences, The George Washington University, Washington, DC Associate
- 2002 - 2003 Co-Director of Undergraduate Honors Thesis and Research, Department of Biological Sciences, The George Washington University, Washington, DC

Education

- 1986 Ph.D. Department of Botany, Miami University, Oxford, OH 45056
- 1983 M.En. Institute of Environmental Sciences, Miami University, Oxford, OH 45056
- 1981 B.S. Natural Systems Department, The Defiance College, Defiance, OH 43512

Grants and Fellowships

- 2003 - 2005 Co-Principal Investigator, (Co-PIs; Drs. M. Allard, C. Smith, G. Hormiga and P. Herendeen), Research sponsored by the NSF, Title: "Capillary Sequencer for the George Washington University Campus" (\$90,588)
- 2002 - 2004 Co-Principal Investigator (Co-PI; K. Deahl and C. J. Baker, USDA), Research with the USDA, Title: "Molecular mechanism(s) of protection against pathogen invasion in potato." (\$100,000)
- 2000 - 2003 Principal Investigator, National Research Initiative/Competitive Grants Program, Plants Response to the Environment, Title: "A genetic approach to determine the role of *GAD2* in the plant stress response." (CGA# 2001-35100-09930, \$150,000)
- 2000 - 2001 Principal Investigator, University Faculty Fund Award, The George Washington University, Title: "Localization of neurotransmitter receptors in plants." (\$11,286)

- 1999 - 2002 Principal Investigator, Research Agreement with Emerald Bio Corporation (formerly Auxein Corporation), Title: Characterization of putative ligand-gated ion channels in higher plants.” (\$40,000 plus funding for the salary and benefits for a postdoctoral associate, Dr. Ganesh Panta, for 7 months)
- 1992 - 1994 Principal Investigator, National Research Initiative/Competitive Grants Program, Plant adaptation to iron deficiency stress. (\$100,000)
- 1985 - 1986 Dissertation Fellow, Miami University,
- 1986 Graduate Enrichment Grant, Miami University
- 1986 Grant-in-Aid of Research, Sigma Xi, Miami University
- 1985 Graduate Students' Achievement Fund, Miami University

Honors

- 1994 Certificate of Merit, USDA, Climate Stress Laboratory, Beltsville, MD
- 1993 Distinguished Service Award, Mid-Atlantic Plant Molecular Biology Society
- 1989 Certificate of Merit, USDA, Plant Molecular Biology Laboratory, Beltsville MD

Committees and Service

- 2003 Organizer of the Twentieth Annual Meeting of the Mid-Atlantic Plant Molecular Biology Society, served on the Program, Abstract, Local Arrangements and Registration committees
- 2002- present Treasurer of the Mid-Atlantic Plant Molecular Biology Society
- 2002 Nineteenth Annual Meeting of the Mid-Atlantic Plant Molecular Biology Society, served on the Program and Registration committees
- 2001 Co-Organizer of the Eighteenth Annual Meeting of the Mid-Atlantic Plant Molecular Biology Society, served on the Program and Registration committees
- 2000 Seventeenth Annual Meeting of the Mid-Atlantic Plant Molecular Biology Society, served on the Program, Registration, Fund Raising, Publicity and Mailing committees
- 1998 Co-Organizer of the Fifteenth Annual Meeting of the Mid-Atlantic Plant Molecular Biology Society, served on the Program, Registration, Fund Raising, Publicity and Mailing committees
- 1997 Co-Organizer of the Fourteenth Annual Meeting of the Mid-Atlantic Plant Molecular Biology Society, served on the Program, Registration, Abstract, Travel Arrangements, Publicity and Mailing committees
- 1996 Co-Organizer of the Thirteenth Annual Meeting of the Mid-Atlantic Plant Molecular Biology Society, served on the Program, Registration, Abstract, Publicity and Mailing committees
- 1995 Co-Organizer of the Twelfth Annual Meeting of the Mid-Atlantic Plant Molecular Biology Society, served on the Program, Registration, Fund Raising, Publicity and Mailing committees
- 1994 Co-Organizer of the Eleventh Annual Meeting of the Mid-Atlantic Plant Molecular Biology Society, served on the Program, Registration, Abstract, Travel Arrangements, Local Arrangements, Publicity and Mailing committees
- 1994 Coordinator of the Climate Stress Laboratory Move, coordinated (planned, designed and organized) the move of six scientists, their staff, chemicals, equipment and furniture into laboratory and office space.

Grant Reviews

National Science Foundation, Integrative Plant Biology
National Research Initiative, Nitrogen Fixation and Nitrogen Metabolism
National Research Initiative, Plant Responses to the Environment
BARD, Field and Garden Crops
BARD, Cellular and Molecular Biology in Agriculture
European Union, Molecular Biology
Jeffress Memorial Trust, Molecular and Cellular Biology

Journal Reviews

Plant Physiology
Plant Growth Regulation
Phytochemistry
Physiologia Plantarum
Photobiology

Professional Organizations

International Society for Plant Molecular Biology
American Society of Plant Biologists
Mid-Atlantic Plant Molecular Biology Society
Mid-Atlantic Section of American Society of Plant Biologists

Invited Lectures

- 2000 Glutamate and GABA-like receptors in Arabidopsis, Symposium, Annual Meeting of the Mid-Atlantic Plant Molecular Biology Society, Beltsville, MD
- 2000 A genetic approach to decipher the biological function(s) of homologues to amino acid neurotransmitter receptors in plants, The Institute for Genomic Research, Rockville, MD
- 2000 Neurotransmitter-like receptors in Arabidopsis, Mini-Symposium, University of Maryland, College Park, MD
- 1999 Evidence that some amino acids are signaling molecules in plants. Towson University Towson, MD
- 1999 Glutamate and GABA: Simply amino acids or signaling molecules in plants? Center of Agricultural Biotechnology, University of Maryland, College Park, MD
- 1998 Role of glutamate and GABA in plants, Department of Biological Sciences, University of Maryland, College Park, MD
- 1998 Thigmomorphogenesis and signaling in plants, or plant involvement in contact sports, Department of Plant and Soil Sciences, Delaware University, Newark, DE
- 1998 Touch responses in plants, Department of Biological Sciences, The George Washington University, Washington, DC
- 1997 Utilization of Arabidopsis for a molecular and genetic approach to study plant responses to environmental stress, Salinity Laboratory, USDA, Riverside, CA

Dissertation or Thesis Committees

2001- present	Dinanet Giraldo, M.S.	BiSc, GWU (Reader)
2002- Present	Paul Kriebel, Ph.D.	BiSc, GWU
2000 - Present	Adeah Pajooohesh-Ganji, Ph.D.	BiSc, GWU

2000 – present	Meliha Dzirlo, M.S.	BiSc, GWU (Reader)
2000 - 2001	Mohammad Nazarian, M.S.	BiSc, GWU
2000 – present	Tulin Olcum, Ph.D.	BiSc, GWU
2000 - 2001	Simin Assadi, Ph.D.	BiSi, GWU (Reader)
1999 – present	Andriana Papaconstantinou, Ph.D.	BiSc, GWU
1999 – 2001	Lori Clow, Ph.D.	Genetics, GWU
1998 – 2002	Konstantina Karyotou, Ph.D.	BiSc, GWU (Reader)

Comprehensive Exam Committees

2000	Hui Li	Genetics, GWU
2000	Keith Edgeman	Genetics, GWU
2000	Hyun-Soo Je	Genetics, GWU
2001	Hui Li	Genetics, GWU
2001	Michelle Mintz	Genetics, GWU
2001	Elizabeth Flynn	Genetics, GWU
2001	Mohammad Nazarian	Genetics, GWU
2001	Erich Boger	Genetics, GWU
2001	Marybeth Daucher	Genetics, GWU

Graduate Student Research

2003 – present	Amir Pooyan Faghfoory, M.S.	Genetics, GWU (Lab rotation/Advisor)
2003 – present	Brandee Price, M.S.	Genetics, GWU (Lab rotation/Advisor)
2003 – present	Siva Balasubramanian, Ph.D.	BiSc, GWU (Advisor)
2001 – 2003	Yakup Batlevi, M.S.	Genetics, GWU (Lab rotation/Advisor)
2000 – present	Jiman Kang, Ph.D.	BiSc, GWU (Advisor)
2000 – 2003	Ross Katkowski, M.S.	BiSc, GWU (Advisor)
2002 – present	James Lam, B.S.	BiSc, GWU (Data based mining)

Undergraduate Research

2003 – present	Megan Martin	BiSc, GWU	<i>AtGLR1.1</i> knockout
2003 – present	Sophia Rafiqi, B.S.	BiSc, GWU	<i>AtGLR1.1</i> promoter
2003 – present	Kelly Lagor, B.S.	BiSc, GWU	GABA permease
2002 – 2003	Michelle Jacobs, B.S.	BiSc, GWU	Truncated <i>GAD2</i>
2002 – 2003	Jacquie Collura, B.S.	BiSc, GWU	<i>GABAperm</i> KO
2001 – 2003	Sohum Mehta, B.S.	BiSc, GWU	DNA-tag mutants, <i>AntiAtGLR1.1</i>
2000 – 2002	James Lam, B.S.	BiSc, GWU	Database mining
2000 – present	Justin Steinkamp, B.S.	BiSc, GWU	Mutant screens
2000	Tessa Humphries-Brickley, B.S.	BiSc, GWU	Immunoblot analysis
1999 – present	Rathai Anandanadesan, B.S.	BiSc, GWU	Mutant screens, NADP-GDH
1999 – 2000	Deepa Ganachari, B.S.	BiSc, GWU	Immunoblot analysis

Technician, Postdoctoral and Scientist Training

2002 - 2003	Jaba Mukhopadhyay	Postdoctoral Research Assistant
1999 - 2000	Ganesh Panta	Postdoctoral Research Assistant

1995 - 1999	Tung K. Fang, Ph.D.	Support Scientist
1997 - 1999	Geraldine Glover, M.S.	Technician
1992 - 1999	Michael McMahon, B.S.	Technician/Support Scientist
1997 - 1999	Elizabeth Orlandi, M.S.	Technician/Support Scientist
1998	Florence Simonet, B.S.	Visiting Student, Paris, France
1998	Caroline Mevel, B.S.	Visiting Student, Paris, France
1997	Cecile Petit, B.S.	Visiting Student, Paris, France
1997	Jaime Ireland, B.S.	Technician (Summer-hire)
1996 - 1997	Aaliya Khan, B.S.	Technician (Summer-hire)
1991 - 1997	Sona Thakkar, M.S.	Technician
1993 - 1994	Ralph Dashner, M.S.	Technician
1994	Robert Donaldson, Ph.D.	Visiting Professor George Washington University
1992 - 1994	Marcia Holden, Ph.D.	Postdoctoral Research Assistant

Mentor for Eleanor Roosevelt High School Internship Program

1998 - 1999	Schaneil Turnbull	Light activation of glutamate decarboxylase
1998 - 1999	Nonyreem Nwaneri	Light activation of NADP(H)-glutamate dehydrogenase
1997 - 1998	Michael Hayden	Identification of Arabidopsis expressing sense or antisense <i>GDH1</i> constructs
1997 - 1998	Vivianne Njoku	Identification of Arabidopsis expressing antisense <i>GAD2</i>
1996 - 1997	Roxanna Diaz	Effect of drought on NAD(H)-glutamate dehydrogenase activity in <i>Arabidopsis thaliana</i>
1995 - 1996	Aaliya Khan	Glutamate dehydrogenase isoenzyme patterns in <i>Arabidopsis thaliana</i>
1995 - 1996	Jessica Eng	The effects of different carbon and nitrogen sources and stress on free amino acid pools in <i>Arabidopsis</i>
1993 - 1994	Maria McGlew	Thigmomorphogenesis in Arabidopsis: A plant's response to touch
1993 - 1994	Clare Deming	Identification of NAD(H)-glutamate dehydrogenase in <i>Arabidopsis thaliana</i> using PCR
1992 - 1993	Mahsa Modarres	Soybean transformation by electrophoresis
1992 - 1993	Jaime Ireland	Soybean transformation by electrophoresis

PUBLICATIONS

Journal Articles

Kang, J. and Turano, F. J. (2003) The putative glutamate receptor 1.1 (AtGLR1.1) functions as a regulator of carbon and nitrogen metabolism in *Arabidopsis thaliana*. Proc. Nat. Acad. Sci. USA 100: 6872-6877.

Turano, F. J., Muhitch, M. J., Felker, F. C. and McMahon, M. B. (2002) The putative glutamate receptor 3.2 from *Arabidopsis thaliana* (AtGLR3.2) is an integral membrane peptide that accumulates in rapidly growing tissues and persists in vascular-associated tissues. Plant Sci. 163:43-51.

Lacombe, B., Becker, D., Hedrich, R., DeSalle, R., Hollmann, M., Kwak, J. M., Schroeder, J. I., Le Novere, N., Nam, H.-G., Spalding, E. P., Tester, M., Turano, F. J., Chiu, J. and Coruzzi, G. M. (2001) On the identity of plant glutamate receptors. Science 292:1486-1487.

Turano, F. J., Panta, G. R., Allard, M. W. and van Berkum, P. (2001) The putative glutamate receptors from plants are related to two superfamilies of animal neurotransmitter receptors via distinct evolutionary mechanisms. Mol. Biol. Evol. 18:1417-1420.

Coleman, S. T., Fang, T. K., Rovinsky, S. A., Turano, F. J. and Moye-Rowley, W. S. (2001) Expression of a glutamate decarboxylase homologue is required for normal oxidative stress tolerance in *Saccharomyces cerevisiae*. J. Biol. Chem. 276:244-250.

Kinnersley, A. M. and Turano, F. J. (2000) Gamma-aminobutyric acid (GABA) and plant responses to stress. Crit. Rev. Plant Sci. 19:479-509.

Turano, F. J. and Muhitch, M. J. (1999) Differential accumulation of ferredoxin- and NADH-dependent glutamate synthase activities, peptides, and transcripts in developing soybean seedlings in response to light, nitrogen, and nodulation. Physiol Plant 107:407-418

Turano, F. J. (1998) Characterization of mitochondrial glutamate dehydrogenase from dark-grown soybean seedlings. Physiol. Plant. 104:337-344

Turano, F. J. and Fang, T. K. (1998) Characterization of two glutamate decarboxylase cDNA clones from *Arabidopsis thaliana*. Plant Physiol. 117:1411-1421

Turano, F. J., Dashner, R., Upadhyaya, A., Caldwell, C. R. and Baughan, G. (1998) Characterization of the glutamate dehydrogenase isoenzyme system in germinating soybean. Plant Sci. 135:137-148

Caldwell, C. R., Turano, F. J. and McMahon, M. B. (1997) Identification of two cytosolic ascorbate peroxidase cDNAs from soybean leaves and characterization of their products by functional expression in *E. coli*. Planta 204:120-126

Turano, F. J., Kramer, G. F and Wang, C. Y. (1997) The effect of methionine, ethylene and polyamine catabolic intermediates on polyamine accumulation in detached soybean leaves. *Physiol. Plant.* 101:510-518

* **Turano, F. J., Thakkar, S. S., Fang, T. K. and Weisemann, J. M. (1997)** Characterization and expression of NAD(H) dependent glutamate dehydrogenase genes in *Arabidopsis thaliana*. *Plant Physiol.* 113:1329-1341

* **Turano, F. J., Dashner, R., Upadhyaya, A. and Caldwell, C. R. (1996)** Purification of mitochondrial glutamate dehydrogenase from dark grown soybean seedlings. *Plant Physiol.* 112:1357-1364

* **Turano, F. J. and Kramer, G. F. (1993)** Effect of metabolic intermediates on the accumulation of polyamines in detached soybean leaves. *Phytochem.* 34:959-968

* **Turano, F. J., Weisemann, J. M. and Matthews, B. F. (1992)** Identification and expression of a cDNA clone encoding aspartate aminotransferase in carrot. *Plant Physiol.* 100:374-381

* **Turano, F. J., Wilson, B. J. and Matthews, B. F. (1991)** Rapid purification and thermostability of the cytoplasmic aspartate aminotransferase from carrot suspension cultures. *Plant Physiol.* 97:606-612

Turano, F. J., Wilson, B. J. and Matthews, B. F. (1990) Purification and characterization of aspartate aminotransferase isoenzymes from carrot suspension cultures. *Plant Physiol.* 92:587-594

Turano, F. J., Jordan, R. L. and Matthews, B. F. (1990) Immunological characterization of *in vitro* forms of carrot homoserine dehydrogenase from carrot suspension cultures. *Plant Physiol.* 92:395-400

Turano, F. J., DeBonte, L. R., Wilson, K. G. and Matthews, B. F. (1987) The cytochrome oxidase subunit II gene from carrot contains an intron. *Plant Physiol.* 84: 1074-1079

Book Chapters

Reardon, E. M., **Turano, F. J.**, Wilson, B. J., Weisemann, J. M. and Matthews, B. F. (1989). Amino acid biosynthesis and nitrogen assimilation in higher plants. in *Physiology, Biochemistry, and genetics of Nongreen Plastids* (Boyer, C. D., Shannon, J. C. and Hardison R. C., eds) pp130-140, The American Society of Plant Physiology.

Matthews, B. F., Reardon, E. M., **Turano, F. J.** and Wilson, B. J. (1988). Amino acid biosynthesis in plants: Approaching an understanding at the molecular level. *Plant Molecular Biology Reporter* 6: 137-154

Patent Applications

Patent submission case # AUX-008-PCT-7224-38 Plant ligand-gated ion channels (Filed 03/2/00), Inventors Kinnersley, A. M. and Turano, F. J.

Patent submission #7224-41/AUX-011-PROV Methods for regulating plant GABA production. (Filed 11/07/00), Inventors Kinnersley, A. M. and Turano, F. J.

Patent docket # 0132.99 A Plant Autophagy Gene (Filed 10/19/00), Inventors Baker, C. J., Deahl, K., Orlandi, E., and Turano, F. J.

Patent submission case # 7224-57:EMB-001-134674 Plant ion channel and methods (Filed 07/20/01), Inventors Kinnersley, A. M. and Turano, F. J.

Published Abstracts

Turano, F. J., Panta, G. R., Allard, M. W. and van Berkum, P. "Genetic and pharmacological evidence for the presence of GABA-like receptors in *Arabidopsis thaliana*." Plant Biology Meeting Providence, RI (08/01)

Turano, F. J. (1999) Differential Expression of Putative Ligand-gated ion channels in *Arabidopsis thaliana*. Final program of the Annual Meeting of the American Society of Plant Physiologists p. 47

Turano, F. J. and T. Fang (1998) Characterization and expression of NADP(H)-glutamate dehydrogenase in *Arabidopsis thaliana*. Abstracts of the 9th International Conference on Arabidopsis Research p. 117

Turano, F. J. and T. K. Fang (1998) Characterization and expression of glutamate decarboxylase cDNA clones in *Arabidopsis thaliana*. Final program of the Annual Meeting of the American Society of Plant Physiologists p. 118

Turano, F. J., and Muhitch M. J. (1997) Accumulation of ferredoxin- and NADH-dependent glutamate synthase activities, peptides and transcripts in developing soybean seedlings in response in response to light and nitrogen. FASEB J.

Turano, F. J., Thakkar, S. S., Fang, T. and Weisemann, J. M. (1996) Characterization and expression of NAD(H)-dependent glutamate dehydrogenase genes in *Arabidopsis thaliana*. Thirteenth Annual Meeting of the Mid Atlantic Plant Molecular Biology Society p. 30

Turano, F. J., Thakkar, S. S. and Fang, T. (1996) Biochemical and molecular characterization of glutamate decarboxylase in *Arabidopsis thaliana*. WASPP Spring meeting

Saunders, J. A., Turano, F. J. and Burek, C. (1994) HPLC analysis of soluble amino acids in plant extracts by stable derivatization using 6-aminoquinolyl-n-hydroxysuccinimidyl carbamate. Phytochemical Society of North America

Donaldson, R. P., Holden, M. J. and Turano, F. J. (1994) NADPH:Ferric chelate reductase induction in the roots of iron deficient tomato plants. WASPP Spring meeting

Turano, F. J. and Thakkar S. (1993) Transformation of soybean by direct uptake. Mid-Atlantic Plant Molecular Biology Society Meeting p. 49

Jordan, R. and Turano, F. J. (1993) Further evidence that pepper mottle and potato virus Y are distinct potyviruses: serology and nucleotide sequence of the coat protein gene and 3' untranslated region of pepper mottle virus NC165. IVth International Congress of Plant Pathology

Turano, F. J. and Upadhyaya, A. (1993) Characterization of glutamate dehydrogenase isoforms from germinating soybeans. Plant Physiol S102: 54

GenBank Submissions

Turano, F. J., Thakkar, S. S. and Muhitch, M. J. (1998) *Glycine max* ferredoxin-dependent glutamate synthase (*GLU*) mRNA. (ACC # AF039851)

Turano, F. J., Thakkar, S. S. and Fang, T. K. (1997) *Arabidopsis thaliana* glutamate decarboxylase (*GADI*) gene, 3' UTR. (ACC # AF060094)

Turano, F. J. (1997) *Arabidopsis thaliana* putative GABA permease mRNA (Bankit # 134810)

Turano, F. J., Caldwell, C. R. and McMahon, M. (1997) *Arabidopsis thaliana* glutathione peroxidase 2 (*GPx2*) mRNA (ACC# U94495)

Turano, F. J., Caldwell, C. R. and McMahon, M. (1996) Soybean ascorbate peroxidase 2 (*APx2*) mRNA (ACC# U56634)

Turano, F. J. and Thakkar, S. (1996) *Arabidopsis thaliana* glutamate decarboxylase 2 (*GAD2*) mRNA (ACC# U46665)

Turano, F. J., Thakkar, S. and Weisemann, J. M. (1996) *Arabidopsis thaliana* glutamate dehydrogenase 2 (*GDH2*) mRNA (ACC# U56635)

Turano, F. J., Turano, F. J., Thakkar, S. and Weisemann, J. M. (1995) *Arabidopsis thaliana* glutamate dehydrogenase 1 (*GDH1*) mRNA (ACC# U37771)

Turano, F. J., Weisemann, J. M. and Matthews, B. F. (1992) Carrot cytoplasmic aspartate aminotransferase (*AAT1*) (ACC# M92660)